Matoušek Award 2019
Final Report

Awardee:	Rajan Iyyappan
Title of the project:	Understanding the regulation of translational reprogramming in the meiosis and mitosis pre and post fertilization
Laboratory:	Laboratory of Biochemistry and Molecular Biology of Germ Cells
Job title:	Post-Doctoral Fellow

Introduction:

In the absence of transcription, the regulation of gene expression in oocytes is controlled almost exclusively at the level of transcriptome and proteome stabilization and at the level of protein synthesis. The stored Specific subset of mRNAs guides meiotic progression, oocyte-embryo transition, and early embryo development. Here we proposed

1. To understand the regulation of translation in meiotic resumption, the oocyte-embryo transition, and early embryo development
2. To understand the major pathway which controlled the oocytes and embryo development
3. And finally explore the gene which is important for the different cell cycle stages.

Principal results:

1. Cells used in this study

   Different stages of the oocytes and embryo were used in this study to address the above mentioned aim. The stages of the cells are GV, MII, Zygote, Zygote metaphase, 2-cell, and 2cell mataphase.

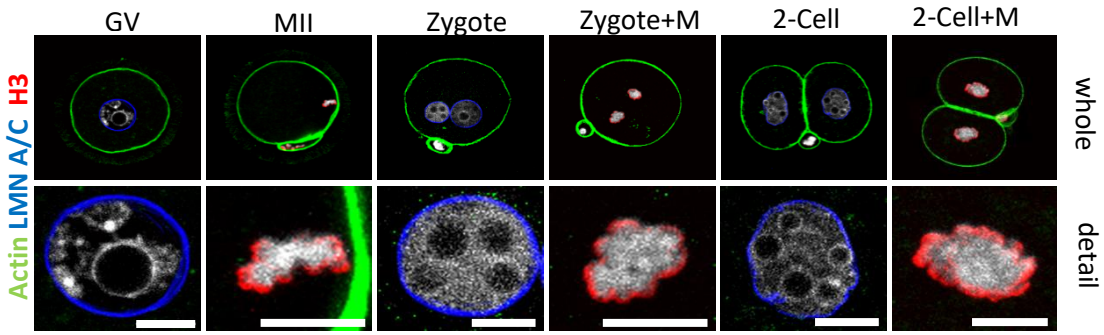

Figure.1. Invitro matured oocytes and embryo were fixed and immunofluorescence staining for actin (green), kinetochores (red) and LMN(blue) was performed.

2. Global translation

Initially we checked the rate of global protein systhesis in these stages. We obeserved the global protein synthesis are decreased in M-Phase compared to interphase as evidensed by S35 methionine labelling and proximate ligation assay.

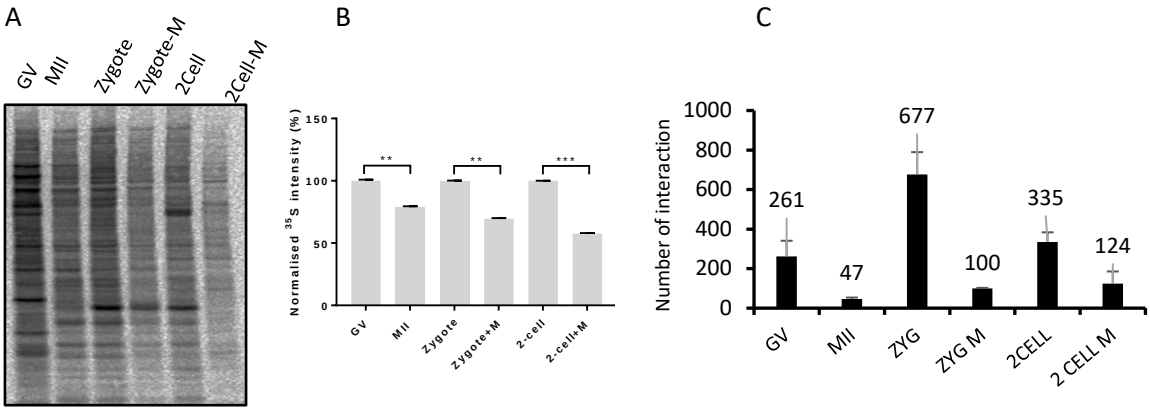

Figure.2. (A). Images of ^{35}S-Methionine incorporation in oocytes and embryo and (B) its quantification. (C) Proximity ligation assay (PLA) detects interaction of two translational components RPL24 and RPS6 in the oocyte and embryo.

3. Cap dependent tanslation and mTORC1

Since these cells purely depend on the translation firstly, we checked the important key protein of the translation initiation and elongation. Interestingy we found that the translation initiation key protein 4E-BP1 regulated differently in M-Phase and interphase. Similar results also observed in the eEF2, one of the rate limiting factor in the translation elongation (Figure 3A). These results indicate the cap dependent tranlsation is active in the M phase. Second, we checked the mTORC1 signalling pathway in thses stage of the cells. Notably, we found the the mTORC1 is active in meiosis, however less active in mitosis (Figure 3B).

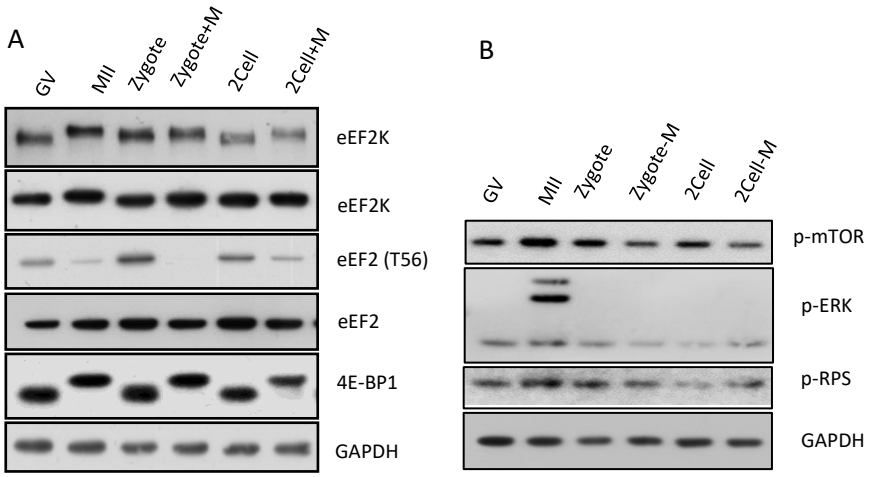

Figure 3. Western blot analysis of (A) translational key proteins in oocytes and embryo (B) mTORC1 signalling protein. GAPDH was used as a loading control.

4. Translational reprogramme

Further deeper anlaysis through polysome bound RNA-seq data show that the transaltion is reprogrammed in 2-cell stages and aslo found the translation rate is high in meiosis than mitosis and oocytes than early embryo

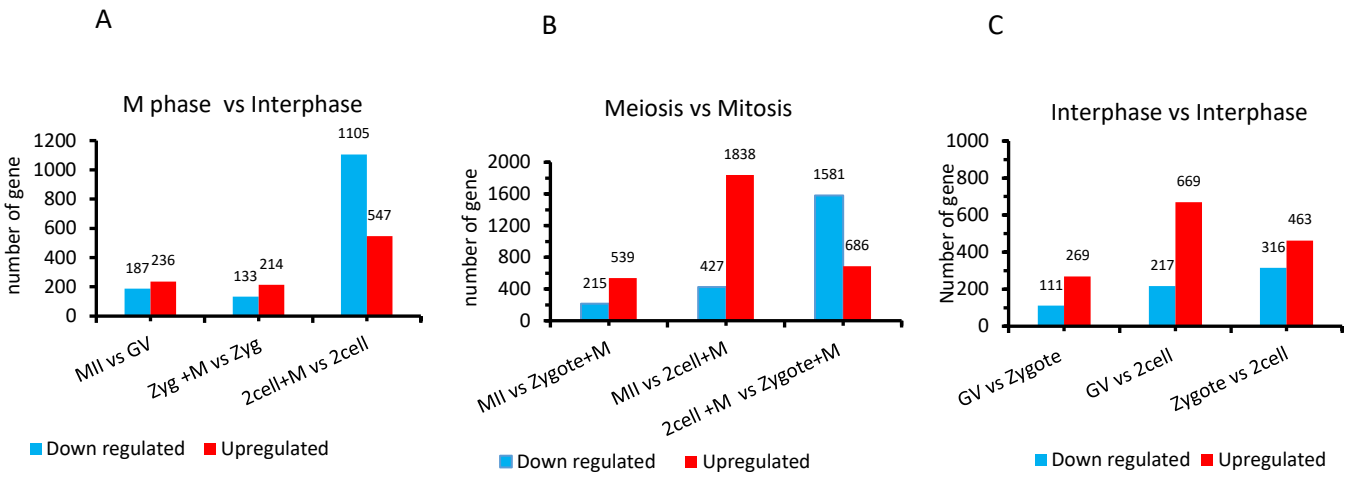

Figure 4. Total number of genes actively involved translation in (A) M-pahse and interphase (B) Meiosis and mitosis (C) oocytes interphase and embryo interphase.

5. Genes important for M phase of oocytes and embryo

Our RNAseq data show that cdc20, nip7, cenp-a and h2afz is consistantly upregulated and elob1 and ooep are downregulated in M – Phase of oocytes and embryo. This indicates the these genes are very important for oocytes and early embryo development.

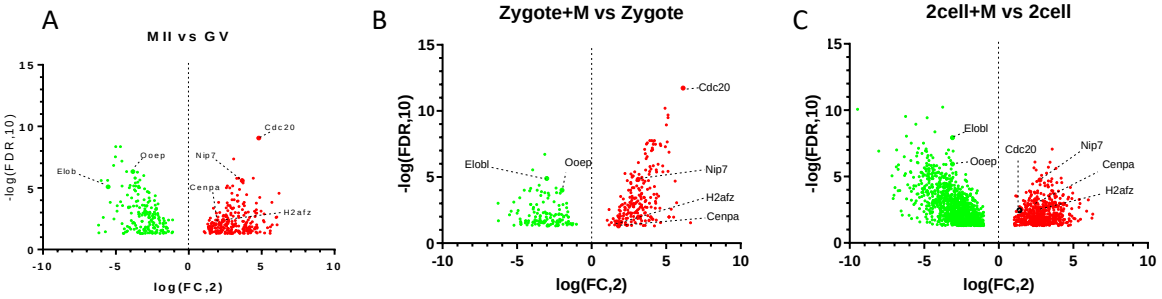

Figure 5. Volcano plot representing up (red) and down (green) regulated genes in different stages of the oocytes and embryo

Conclusions:

- Global protein synthesis are decreased in meiosis and mitosis
- Translational reprogram occurs at 2nd mitosis
- Translational rate is higher in meiosis than in mitosis
- Translational rate is higher in oocyte than in early embryo
- Cap-dependent translation is active in meiosis and mitosis
- mTORC1 dependent translation is more active in meiosis, less active in mitosis
- cdc20, nip7, cenp-a and h2afz (up) and ELOB1 and ooep (down) genes are important for oocytes and early embryo.

Future work:

We are trying to explore more in detail for calculating the translational efficiency and how its regulated in these stages of the oocytes and early embryo through bioinformatics approach. Soon after this we will publish theses finding in good reputed international journal.

Financial and budget report: Attached with this report as excel file

Date: 10.05.2021 Awardee: Rajan Iyyappan .....

Date: 10.05.2021 Head of the Lab: Andrej Susor .....

Comments from the head of the lab:

Rajan carried out experiments as he had designed for this project with the help of other lab members. He successfully completed polysome profiling, followed by RNA separation from polysome samples, RNAseq, and preliminary analysis, in addition to biomolecular analysis. To support the hypothesis, he extracted the key genes from the RNAseq results. The biochemistry analysis, in particular, highlights the role of cap-dependent translation in M-phase. He discovered that the global translation is reprogramming at the 2-cell stage, which strengthens the project even further. Overall, he discovered that global translation is regulated differently in oocytes and embryos, as well as in meiosis and mitosis. His polysome results provide a good foundation for researching oocyte and embryo translational changes. We made 3 research publication using the RNAseq data collaborating with national and international lab. We intend to publish the results of this whole project in a reputable international journal soon.

In conclusion, I believe that the work of Rajan met the principal aims of the Dr. J. Matoušek Award.

Dr. J. Matoušek Award IAPG CAS

Call 2018

Attachment to Final Report

Name and surname of the PI: **Rajan Iyyappan**

	Planned costs in CZK	Real costs in CZK	Justification
1. CONSUMABLES (Subtotal)	**0**	**63898**	I didn't subit the planned cost at the time of application
1.1. Costs for material (e.g. Lab material - non-investments only)		**63898**	All reagents are directly used in this project
1.2. Costs for services (e.g. Publication fees, patent application fees)		**0**	
1.3. Travel costs		**0**	
2. PERSONAL COSTS (Subtotal) - (If applicable)	**0**	**22000**	
2.1. Personal costs (DPP/DPČ)**		**22000**	salary
2.2. Costs for social and health insurance		**0**	
3. TOTAL	**0**	**85898**	

*Please, fill in the white boxes only according to the needs of your project.

**If applicable, please, include in the description type of contract (DPČ/DPP) and number of spent working on the project.

qPCR Master mix	16771
Primer	1436
Restriction enzyme	2698
IVT kit	11000
Culture Media	1470
RNA ladder -Marker	15221
96 well plates	15302
Total cost for reagents	63898